Molecular Biology

ISOLATING DNA FROM *VAGINA WASH*

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Purity and quantity of DNA isolated is very important for further sequencing and amplification. It has been reported that gram negative bacteria were responsible for the endotoxin/lipopolysaccharide (LPS) found in premature birth victims. Later on, it was noticed that many premature birth victims with high level of endotoxin/lipopolysaccharide, tested negative for traditional test for gram negative bacteria. The aim of this study is to provide evidence about the existence of *provetella*, an anaerobic gram negative bacteria in the samples collected from this group of patients. It also aims to compare the amount of *provetella* with other gram negative bacteria (E.coli) for which other patients are tested positive and to establish *provetella* as the major source of endotoxin production. High quality pure DNA was isolated from vaginal washes in a high enough quantity to sequence and amplify via polymerase chain reaction (PCR). It is important to isolate high quality pure DNA in order to prevent the samples' proteins from interfering with the enzymes used for further reactions. The PCR will make available sufficient DNA for testing, enough to quantify the amount of cells in order to tract the initial amount of *provetella* in the samples. Vagina washes were obtained from 16 women using 5 ml of saline (0.9%sodium chloride/ Irrigation USP Abbot Laboratory lot# 08-038-48-1) flushed into the vagina. The collected sample contained vaginal epithelial and bacterial cells based on the site where the sample were collected. The wash was centrifuged (microcentrifuge Eppendorf) at 6000g x 10 min tow times and the cells were harvested after discarding the supernatant. Pellet (cells) were immediately used for DNA isolation. The DNA was extracted from the cells, using Bactozol™ kit Cat. No BA 154 (Molecular Research Center, INC; Cincinnati, Ohio). The amount of isolated DNA was measured using BECKMAN DU-600 spectrophotometer at wavelength 260nm, and the purity was determined by ratio of Absorbance readings at 260/280nm. All steps of extraction were performed within 1.5 hour. The DNA concentration of more than 80% of samples was between 6 and 81µg/ml. Other samples had DNA concentration of $\geq$ 4µg/ml. 75% of the samples had purity rate between 1.8 and 2.33, with a mean average of 2.00 $\pm$ 0.22 (n=16). Bactozol™ kit performed more efficiently than other comparable kits for isolating pure DNA. This pure DNA has been stored in ultra pure water at -86^0 C and will later be sequenced and analyzed by PCR.